Joint experimental and theoretical investigation of the propensity of peptoids as drug carriers

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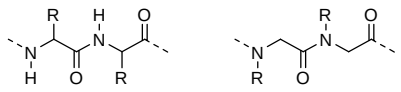
Abstract: - Transport across the membrane is one of the key obstacles drug molecules must overcome to effectively function in the cell. Potential drugs should therefore be designed taking into account these specific membrane-transport properties. Here we investigate, both experimentally and by simulation, one promising class of molecules. Peptoids are structural analogs of the amino acids that make up the proteins of the cell. They constitute a very promising class of drug carriers because they are known to cross several biological barriers and, unlike proteins, they are unaffected by proteases. All-atom molecular dynamics simulations demonstrate that even short peptoid molecules exist in a rapidly fluctuating conformational ensemble, which differentially presents hydrophobic and hydrophilic molecular surface area to its environment, making these molecules well suited candidates for tunable membrane transport. *In vivo* studies showed that the synthesized peptoid Fluo-{6,6,6,6,6,6}-NH_2 efficiently translocates into the cells and accumulates preferably in the cytosol.

Key-Words: - drug carrier, peptoid, membrane transport, specific accumulation, cytosol, molecular surface area, all-atom simulation

1 Introduction

It is now well established, that peptides bearing basic amino acid residues are taken up rapidly by cells in culture.[1-9] These peptides can directly traverse the plasma membrane by a today unknown mechanism, independent of classical receptor-mediated pathways. It turned out that basic domains in the structure of the peptides were mainly responsible for the translocation of naturally transduced proteins and were therefore called protein transduction domains (PTDs).[10-12] They transport covalently attached cargo molecules of diverse chemical nature (oligonucleotides, proteins, fluorophores and even liposomes or nanoparticles) into cells. Peptides composed of α–amino acids (**1**, Fig. 1) with the same properties were discovered or designed since then, and are described as a functional group by the term cell-penetrating peptides (CPPs).[10-12]



1 **2**

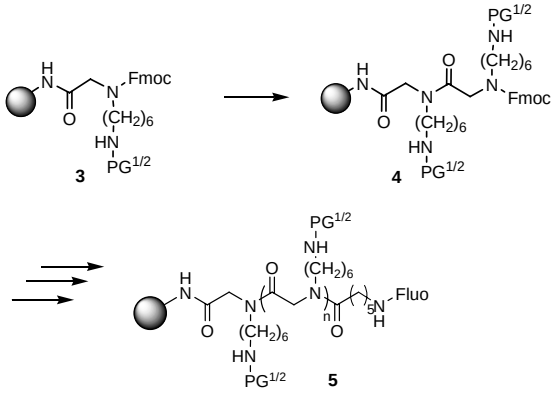
Fig 1. Structures of the backbone of an α-peptide (**1**) and a peptoid (**2**).

Among the most important structural features for cellular uptake efficiency of CPPs one can cite short size, high content of cationic residues, and variable spacing between the charges. The backbone conformation does not seem to play a critical role.[10-12] However, CPPs are usually unstable due to

in vivo proteolysis. This implies that short peptide mimetics with modified backbones, carrying basic functionalities such as amino or guanidinium groups may serve as valuable alternatives to the CPPs because of their enhanced stability *in vivo*.
Peptoids (oligo-*N*-alkylglycines) (**2**, Fig. 1) are stable against proteases, but are usually less prone to aggregation.[3,14,15] There are also several reports on the use of peptoids as effective, water-soluble non-toxic molecular transporters for intracellular drug delivery or as molecular probes for bioconjugation.[16-22]

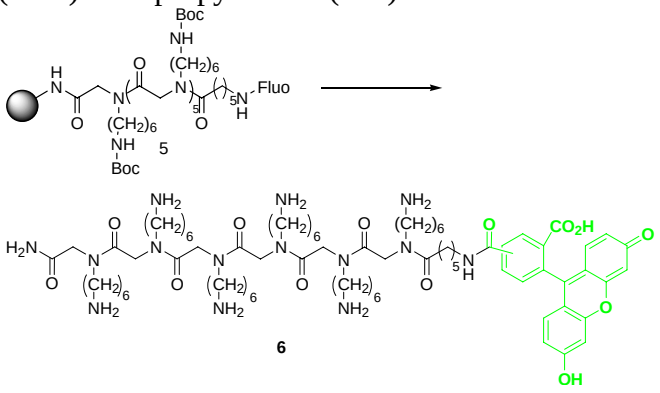
2 Experimental

The building blocks were prepared starting from 1,6-diaminohexane as described in the literature.[22] The protected and functionalized building blocks were assembled using the solid-phase synthesis (Scheme 1).



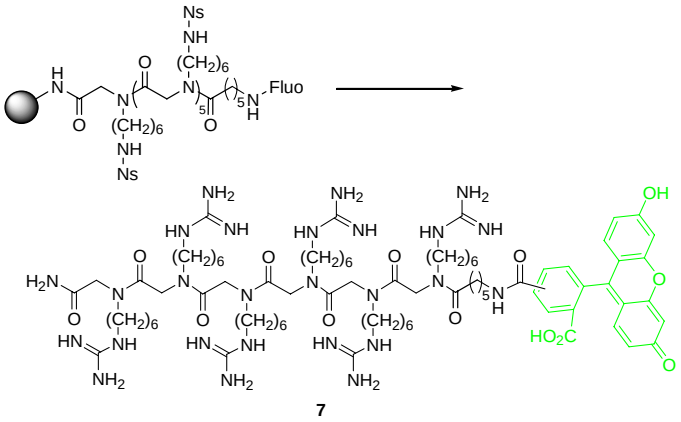
Scheme 1. Solid phase synthesis of peptoids.[21]

After addition of a spacer and fluorescent labeling the amino-peptoid Fluo-{6,6,6,6,6,6}-NH_2 **6** was isolated and simultaneously deprotected and cleaved from the resin, by using trifluoroacetic acid (TFA)/triisopropyl silane (TIS).



Scheme 2. Cleavage of amino-peptoid Fluo-{6,6,6,6,6,6}-NH_2 **6**.

The molecule was characterized by mass spectrometry, UV/VIS and IR. Before the guanidinium-peptoid Fluo-{6^G,6^G,6^G,6^G,6^G,6^G}-NH_2 **7** could be cleaved from the solid support, it had to be orthogonally deprotected and the free amino-side chains were transformed to guanidinium groups. Finally, the guanidinium-peptoid was cleaved from the resin, isolated and characterized following the procedure of **6**.

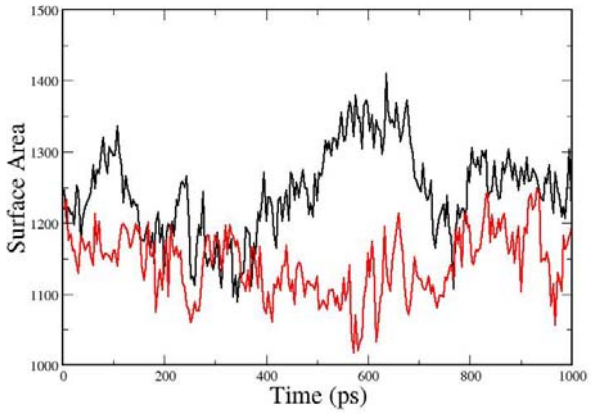


Scheme 3. Deprotection, formation of the guanidinium groups and cleavage from the solid support of guanidinium-peptoid Fluo-{6^G,6^G,6^G,6^G,6^G}-NH_2 **7**.

3 Modelling

To investigate propensity of this molecule for membrane transport the hydrophilic and hydrophobic surface areas must be estimated from realistic models. We have therefore conducted a 1ns molecular dynamics simulation of the peptoid using the General Amber Force Field (GAFF)[23]. The atomic partial charges were assigned according to the AM1-BCC model[24]. The system was solvated in a box with explicit water molecules (TIP3P) and appropriate counterions to neutralize the whole system. The starting conformation was then minimized and slowly equilibrated to the production temperature of 300 K, the simulations were carried out in the NPT ensemble at ambient pressure.

From this simulation we took snapshots every 4ps and extracted the corresponding conformations. The hydrophobic/hydrophilic surfaces of the molecule were then calculated with the program MSMS[25], they are shown in Figure 2.
As can be seen from the surface analysis, the molecule is amphiphilic in a non-trivial fashion, facilitating membrane transport. It fluctuates



between different **Fig. 2:** Time development of the hydrophilic (black) and hydrophobic surface are of the simulated peptide over a 1ns simulation (sampling every 4 ps)

conformational ensembles which exhibit varying hydrophilic and hydrophobic surface areas to their environment, respectively. The conformational flexibility of the molecule permits switching between distinct conformations that permit coexistence with either the hydrophilic environment encountered in aqueous solution or the hydrophobic environment encountered in membranes.

4 Conclusion

We have synthesized the peptoid Fluo-{6,6,6,6,6,6}-NH_2 **6** with amino side chains having both hydrophilic and lipophilic properties and its analog **7** baering guanidinium groups. *In vivo* studies showed that the side chains are crucial of the cell penetrating activity. Furthermore, it turned out that a given side chain favors accumulation in a specific cell region. Finally, low cytotoxicity and high stability makes the molecules based on the peptoidic backbone attractive candidates for *in vivo* intracellular drug delivery.

Atomistic simulations confirmed that the molecules are characterized by different surface patches which are differentially in contact with the environment, depending on the conformation. Even in aqueous solution our simulations demonstrate an amphiphilic character of the molecules, which displays nearly equal, but fluctuating amounts of surface area, compatible with aqueous solution and membrane environment. Experimental evidence suggests that the molecules are indeed translocated into the cells. Amino-peptoid **6** accumulates preferentially in the cytosol whereas guanidinium-pepoid 7 resides mainly in the nucleus. Both compounds do not show significant toxicity. [22]

In future investigation we will extend this work by varying length and composition of the peptoid molecules in order to modulate their propensity for membrane transport. Atomistic simulations as those performed above can help guide the design and choice of the many possible molecules towards those that have the highest likelihood of achieving the desired experimental properties.

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